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Stable Isotopic Analysis of Human Diet in the Marianas Archipelago, Western Pacific

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ABSTRACT Proportions of marine vs. terrestrial resources in prehistoric human diets in the southern Mariana Islands (Guam, Rota, Saipan), Micronesia, have been estimated by analysis of stable isotope ratios of carbon and nitrogen in bone collagen and of carbon in apatite. The isotopic composition of marine and terrestrial food resources from the Marianas have also been determined. Experimental evidence shows that collagen carbon isotopes mainly reflect those of dietary protein sources and thus overestimate the contribution of marine animal foods. Marine protein consumption apparently ranges from ~20% to ~50% on these islands. Experiments also demonstrate the carbon isotope ratio of bone apatite carbonate accurately reflects that of the whole diet. Carbonate carbon isotope data suggest some individuals consumed significant amounts of ^{13}C-enriched (C_4) plants or seaweeds. Sugar cane is an indigenous C_4 crop and seaweeds are eaten throughout the Pacific, but they have not been considered by archaeologists to have been prehistoric dietary staples. Apatite carbon isotope analysis has apparently identified previously unrecognized prehistoric dietary adaptations in the Mariana Islands, but this must be confirmed by archaeobotanical evidence. Am J Phys Anthropol 104:343–361, 1997.

Stable isotope analysis has proven useful for reconstructing human diets in tropical and subtropical coastal settings where both marine and terrestrial resources were exploited (Keegan and DeNiro, 1988; Norr 1995; Schoeninger et al., 1983; Sealy and van der Merwe, 1988; Walker and DeNiro, 1986). Reconstructing the diets of prehistoric Chamorro peoples of the Marianas Archipelago with stable isotope ratios of bone collagen should be simple because they occupy small, comparatively simple ecosystems in which most terrestrial dietary resources have low $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios and most marine resources have high ratios. Stable isotope analysis of their bone collagen can be used measure variation in proportions of marine versus terrestrial dietary resources. It should thus be possible to evaluate relationships between diet and age, gen-

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der, status and health, determine if diachronic trends exist and whether they are associated with population increase, social differentiation, environmental degradation and subsistence change.

Diet reconstruction with stable carbon isotopes is usually predicated on the assumption that all dietary macronutrients contribute equally to carbon in bone collagen (Schwarcz, 1991; Schoeninger, 1989). Recent experiments on the relationship between diet and bone collagen isotopic composition demonstrate that bone collagen carbon is derived mainly from dietary proteins (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). Therefore, where protein and nonprotein resources have different carbon isotope ratios, collagen may not accurately reflect the isotopic composition of the whole diet. In the Marianas, most terrestrial staple foods such as taro, yam, sweet potato (introduced historically), breadfruit and rice (Pollock, 1986; Hunter-Anderson et al., 1995), have very little protein and low $\delta^{13}C$ values. Almost all marine staple foods, which include fish and shellfish, are mostly protein and have high $\delta^{13}C$ values. Therefore isotopic analysis of collagen in prehistoric human bone may provide incomplete and inaccurate information about the whole diet isotopic composition.

The whole diet carbon isotopic composition can, however, be reconstructed from analysis of bone apatite carbonate. Controlled diet experiments with rodents have demonstrated that the carbon isotopic composition of bone apatite carbonate accurately reflects that of the whole diet and does not over- or underestimate the proportions of carbon atoms from fats, carbohydrates and proteins (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). Isotopic analysis of bone apatite carbonate can thus resolve ambiguities in interpretation of collagen carbon isotopes.

Isotopic analysis of bone apatite and collagen carbon isotopes of prehistoric human bones from Guam, Rota and Saipan has revealed dietary adaptations that have not previously been considered in reconstructions of Chamorro diet. If diagenetic alteration of the carbon isotope composition of apatite carbonate can be discounted, these data indicate that sugar cane and/or seaweeds were an important part of prehistoric diets on Saipan, but not on Guam or Rota. Sugar cane and seaweed have been important dietary items of Austronesian-speaking populations elsewhere in the Pacific (Abbott, 1991; Daniels and Daniels, 1993) and may have been systematically exploited by some prehistoric Chamorro populations in the Marianas.

MARIANAS ENVIRONMENTS, ARCHAEOLOGY AND SUBSISTENCE

The Mariana islands are located in the north-western part of Micronesia, almost 2000 km east of the Philippines. Human bones were analyzed from archaeological sites on Guam, Rota and Saipan. Each island has a volcanic core surrounded by plateaus of marine limestone and fringing reefs. Surface water is usually limited to small springs, and streams and wells are common. Rota has a narrow reef and little or no lagoon, but Guam, and especially Saipan, have larger reefs and lagoons in the vicinity of the sites studied. Most prehistoric settlement sites are on or near the coast, but in late prehistoric times there was some inland settlement (Butler, 1988; Hunter-Anderson, 1994).

The indigenous peoples of the Marianas, now known as Chamorro, are the descendants of Austronesian-speaking groups who first colonized these islands around 3500 years ago (Hunter-Anderson and Butler, 1995). Most burials are from contexts which date from 700 A.D. to European contact in the 16th century. The period after 1000 A.D. is usually referred to as the Latte Period, so-called because of the distinctive arrays of stone pillars, called Latte sets, which frequently occur at sites of this period. A Latte set consists of two parallel rows of two to seven stone pillars, originally fitted with capstones. The Latte sets are often assumed to be the bases of wood platforms for raised houses, but may also have served other functions (Hunter-Anderson and Butler, 1995). Latte Period burials are usually clustered around these structures. Mortuary practices were diverse, including simple primary burials, cremations, and burial of skeletal elements from several individuals (Hanson, 1988, 1995; Hanson and Gordon, 1989).

Chamorro subsistence involved exploitation of shellfish, near-shore and deep water

fish such as marlin, swordfish, dolphin fish, mahi-mahi, *Coryphaena* spp., tuna and sea turtles (Butler, 1988; Davidson and Leach, 1988; Amesbury and Hunter-Anderson, 1989; Amesbury et al., 1989). The only terrestrial animal protein sources are coconut crabs, land crabs, fruit bats, a large monitor lizard and several species of birds. Coconut crab (*Birgus latro*) can attain a carpace diameter of 32 cm and often exceeds 3 kg in weight. It has been overexploited in historic times and is considered an endangered delicacy throughout the tropical Indo-Pacific region (Springthorpe, 1991). Land crab has a carpace diameter of up to 15 cm (Grzimek, 1984). Both may have been significant sources of terrestrial protein for prehistoric populations in the Marianas, but they are unlikely to be preserved in archaeological sites. Pig, dog and chicken are common in Polynesia but apparently were not present in the Marianas prehistorically because their bones have not been found in archaeological sites. The largest terrestrial mammal at contact times was the rat (Butler, 1988). The majority of the diet at the time of contact in the 1520s was obtained from starchy tree and root crops (Pollock, 1986). Major indigenous crops include breadfruit, true taro, giant swamp taro, yams, bananas, sugar cane, coconuts and rice. Minor plant foods include Polynesian arrowroot, cycad seeds, pandanus and some fruits. Maize, sweet potatoes, manioc (cassava), papaya, chili peppers, chickens, pigs and deer were introduced by the Spanish after contact (Pollock, 1986; Butler, 1988). Seaweeds are reported to be minor dietary supplements throughout the Pacific (Abbott, 1991) and two species of seaweed are still eaten occasionally in the Marianas. The faunal remains from archaeological sites on Rota are dominated by fish and shellfish with a significant component of large pelagic fish (Butler, 1988; Carucci, 1988; Davidson and Leach, 1988). Faunal samples from sites on the west coast of Saipan, where there are large lagoon areas, contain large amounts of shellfish and the fish are mostly reef and lagoon species (McGovern-Wilson, 1989; Butler, 1995; Hunter-Anderson, 1996).

Archaeological evidence indicates that a significant increase in population occurred in the Marianas beginning sometime after 500 A.D. and reached dramatic proportions after 1000 A.D., during the Latte period (Hunter-Anderson and Butler, 1995; Butler, 1988). At this time significant settlements occurred in interior settings. Major changes in ceramic forms indicate an emphasis on storage and boiling functions, and stone mortars, pounders and pestles become abundant. Dry land rice cultivation is thought to have been introduced in the Latte Period (Hunter-Anderson et al., 1995). The archaeological evidence thus suggests population expansion was accompanied by intensification of agricultural production (Butler, 1988).

Bioarchaeological analyses indicate a generally healthy population throughout most of the Latte Period. Dental caries occur in very low frequencies (0–4.2%) in most archaeological populations, but two populations from Saipan have caries frequencies of 10.2 to 11.0% (Hanson, 1995). There is skeletal evidence of recurring, non-specific chronic infections. On Rota there is some evidence of a suboptimal weaning diet (Hanson, 1988).

FOODWEB STABLE ISOTOPE COMPOSITION

The distribution and variation of foodweb stable isotopes is discussed in detail elsewhere (Ambrose, 1993; Schwarcz and Schoeninger 1991; Pate, 1994) and is only briefly summarized here. Stable isotope ratios are expressed as parts per thousand (‰) relative to a standard reference material (the PDB marine limestone for carbon, and atmospheric N_2 [AIR] for nitrogen) using the delta (δ) notation, where

$$\delta^{13}C_{(PDB)} = \left[\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{PDB}} - 1\right] \times 1000‰$$

$$\delta^{15}N_{(AIR)} = \left[\frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{AIR}} - 1\right] \times 1000‰$$

Most dietary resources have lower $^{13}C/^{12}C$ ratios than PDB and higher $^{15}N/^{14}N$ ratios than AIR, leading to negative $\delta^{13}C$ and usually positive $\delta^{15}N$ values (Table 1).

Carbon isotopes

Carbon isotopes can be used to discriminate between terrestrial plants that fix atmospheric CO_2 by different photosynthetic pathways, named C_4 and C_3, after the number of

TABLE 1. Carbon and nitrogen concentration and isotopic composition of terrestrial and marine foods from Micronesia[1]

Species name	Common name (variety, preparation)	wt. %C	wt. %N	$\delta^{13}C$‰	$\delta^{15}N$‰
Terrestrial Plants					
Musa sp.	Banana (thick-skin, raw)	40.6	0.24	−24.8	[1]
Musa sp.	Banana (thick-skin, boiled)	40.8	0.41	−24.8	[1]
Musa sp.	Banana (thin-skin, raw)	40.6	0.39	−25.8	[1]
Musa sp.	Banana (long green, raw)	40.7	0.65	25.7	[1]
Curcurma longa	Turmeric (raw)	48.8	0.34	−27.7	[1]
Curcurma longa	Turmeric (boiled)	51.3	0.21	−28.0	[1]
Capsicum frutescens	Chili pepper (short red, raw)	51.7	2.01	−31.3	1.5
Capsicum frutescens	Chili pepper (short red, boiled)	51.9	1.92	−27.9	1.7
Capsicum frutescens	Chili pepper (long green, raw)	50.7	2.47	−28.9	6.2
Capsicum frutescens	Chili pepper (long green, boiled)	48.3	2.65	−29.4	5.5
Dioscorea alata	Yam (red-skin, raw)	42.7	0.94	−26.9	4.8
Dioscorea alata	Yam (red-skin, boiled)	42.9	0.64	−27.8	1.9
Dioscorea esculenta	Yam (yellow-skin, raw)	42.9	0.65	−27.2	2.7
Dioscorea esculenta	Yam (yellow-skin, boiled)	41.1	0.49	−26.8	4.5
Dioscorea aculeata	Yam (var. spinosa, raw)	42.1	1.78	−29.3	−0.1
Dioscorea aculeata	Yam (var. spinosa, boiled)	42.7	1.45	−27.2	0.2
Dioscorea aculeata	Yam (var. spinosa, leaves)	45.4	1.22	−30.7	−0.3
Ipomea batatas	Sweet potato (red-skin, raw)	41.7	1.28	−28.4	3.9
Ipomea batatas	Sweet potato (red-skin, boiled)	41.2	0.16	−28.4	[1]
Ipomea batatas	Sweet potato (yellow-skin, raw)	41.8	0.21	−25.5	[1]
Ipomea batatas	Sweet potato (yellow-skin, boiled)	41.9	0.42	−27.4	[1]
Annona squamosa	Sour sop (a mulberry, raw)	44.1	0.96	−29.2	7.8
Artocarpus altilis	Breadfruit (raw)	42.7	0.63	−28.1	4.0
Artocarpus altilis	Breadfruit (boiled)	42.8	0.37	−28.1	[1]
Artocarpus altilis	Breadfruit (raw, fermented)	43.8	0.64	−27.0	6.4
Cordyline terminalis	Hawaiian ti (aerial tuber, raw)	41.9	0.15	−25.8	[1]
Cycas circinalis	Cycad (endosperm flour, leached)	44.0	2.08	−23.1	1.1
Cocos nucifera	Coconut (sap syrup)	39.8	0.06	−24.4	[1]
Colocasia esculenta	Taro (raw)	43.5	0.34	−31.1	[1]
Cyrtosperma chamissonis	Giant swamp taro (raw)	43.2	0.20	−26.9	[1]
Terrestrial Animals					
Pteropus mariannus	Fruit bat (skin)	51.4	14.8	−24.3	6.2
Pteropus mariannus	Fruit bat (flesh)	55.2	12.5	−25.3	5.0
Birgus latro	Coconut crab	48.7	14.7	−24.1	5.9
Cardisoma sp.	Land crab	46.1	11.8	−23.8	8.0
Marine Animals					
Tridacna maxima	Giant clam	45.0	11.3	−15.6	4.3
Makaira sp.	Marlin[2]	47.8	15.4	−15.4	13.0
Panulirus sp.	Spiny lobster	48.1	14.1	−13.2	10.7
Coryphaena hippurus	Dolphin fish[2]	49.7	12.5	−15.7	12.1
Octopus sp.	Octopus	43.4	10.6	−14.6	9.1
Sargocentron sp.		51.1	4.3	−13.9	10.1
Acanthurus sp.	[3]	53.0	13.6	−14.6	9.2
Belonidae	Needlefish	50.5	16.7	−11.3	9.7
Acanthocybium solandri	Wahoo[2]	47.7	15.5	−15.8	12.2
Seaweed					
Gracilaria tsudae	red algae (raw)	30.6	2.6	−15.4	5.2
Gracilaria tsudae	red algae (raw)[4]	36.5	3.8	−15.8	4.8

All samples analyzed are edible tissues. All plant samples were purchased dried in Guam or Yap, except for coconut syrup from Tobi Island, Republic of Palau.
[1] Insufficient nitrogen for isotopic analysis.
[2] Deep water.
[3] Netted.
[4] Same sample, pretreated with 0.2 M HCl to remove carbonates.

carbon atoms fixed in the first stage of photosynthesis. Economically important C_4 plants in the Pacific today include maize (recently introduced) and sugar cane (indigenous). C_3 plants include rice, all root crops, vegetables, nuts and fruits. The ultimate source of carbon in plants is atmospheric CO_2. C_3 plants discriminate more than C_4 plants against atmospheric $^{13}CO_2$. C_3 and C_4 plants fix carbon with average $\delta^{13}C$ values of −26.5‰ and −12.5‰, respectively (Smith, 1972). Atmospheric CO_2 which now has a $\delta^{13}C$ value of about −7.9‰, was less negative by about 1.6‰ in the preindustrial era,

before dilution by C_3 fossil fuel combustion (Marino and McElroy, 1991). Therefore, prehistoric terrestrial foodweb $\delta^{13}C$ values should corrected by this amount. Humidity, temperature, light intensity and other environmental factors may also influence C_3 plant $\delta^{13}C$ values in complex ways, causing small (1–3‰) microhabitat (Tieszen, 1991) and macroregional (van Klinken et al., 1994; van Klinken et al., in press) variations in foodweb carbon isotopic composition.

Carbon in marine environments is ultimately derived from dissolved bicarbonate, which has a $\delta^{13}C$ value of ~0‰. Marine foodwebs are based mainly on plants with the C_3 pathway and have $\delta^{13}C$ values averaging −19‰ (Smith, 1972; Smith and Epstein, 1971). Marine foodwebs thus have carbon isotope compositions intermediate between those based on terrestrial C_3 and C_4 plants. Exceptions include coastal and estuarine ecosystems where estuarine and aquatic C_4 sea grasses, with $\delta^{13}C$ values of around −12‰ and −6‰, respectively, contribute significant amounts of carbon to the foodweb (Schoeninger and DeNiro, 1984; Keegan and DeNiro, 1988). The industrial effect on marine foodweb $\delta^{13}C$ values should be much less because of the greater size and slower turnover of the marine dissolved carbonate reservoir (Broecker et al., 1979). Prehistoric marine foodweb $\delta^{13}C$ values are thus probably only slightly less negative than those of modern ones. We shall use a correction of ~0.5‰ for marine resources, but this is a very crude guesstimate. This effect may be less for pelagic than for near-shore resources.

There is a small stepwise increase in $\delta^{13}C$ values of about 1‰ between trophic levels in both marine and terrestrial ecosystems (Fry and Sherr, 1984; Schoeninger, 1985), but its existence is often questioned because it is so small and difficult to define. Prehistoric East African Neolithic pastoralists, whose diets were likely to have been predominantly milk, meat and blood of C_4-fed cattle and caprines, have the highest known $\delta^{13}C$ values (Ambrose, 1986). The East African Neolithic data are consistent with a trophic level effect in carbon isotopes.

Nitrogen isotopes

Nitrogen isotopes distinguish marine from terrestrial plants and plants with nitrogen fixing symbioses from others. The $\delta^{15}N$ values of marine plants are about 4‰ higher than those of terrestrial ones, and nitrogen fixers, such as legumes, have lower $\delta^{15}N$ values than other plants (Delwiche and Steyn, 1970; Wada et al., 1975). Legumes were not a significant part of prehistoric Chamorro diets, but N-fixation also occurs in other plant families. Tropical reef ecosystems often derive substantial proportions of nitrogen from N-fixing blue-green algae and thus have $\delta^{15}N$ values similar to those of terrestrial ecosystems (Schoeninger and DeNiro, 1984). Algae and sea grasses in the Caribbean, where shallow reef and lagoon environments predominate, have mean $\delta^{15}N$ values of +2.7 and +1.5‰, respectively (Keegan and DeNiro, 1988).

A step-wise enrichment in $\delta^{15}N$ values of 3–4‰ between trophic levels in both terrestrial and marine ecosystems has been observed from plants to herbivores to primary and secondary carnivores (Schoeninger and DeNiro, 1984; Minagawa and Wada, 1984). Nitrogen isotope ratios can thus be used to determine trophic level within terrestrial ecosystems, and proportions of marine to terrestrial resources. There may be climatic and physiological influences on nitrogen isotope variation within and between ecosystems and some of this variation may lead to greater differences between trophic levels (Ambrose, 1991). Climate and water availability were probably similar for all populations analyzed so these influences will be considered constant for our purposes.

DIET-BONE ISOTOPIC RELATIONSHIPS

Diet reconstruction with stable carbon and nitrogen isotopes is predicated on the assumption that you are what you eat. In other words, the isotopic composition of the tissues analyzed is assumed to be a direct and constant function of that of the diet. Where there are significant differences in isotopic composition of major classes of dietary resources, their proportions can be estimated by isotopic analysis of the consumer's tissues (Ambrose, 1993; Schwarcz, 1991).

Experiments designed to evaluate the basic assumptions of diet-tissue carbon isotope relationships (Ambrose and Norr, 1993; Tieszen and Fagre, 1993) now provide the foundation for substantially a revised model of diet reconstruction with carbon isotopes of bone collagen and apatite carbonate. It will be presented in detail here for the first time and applied to the interpretation of dietary adaptations in the Marianas.

Diet-collagen relationship

Carbohydrates and fats have no nitrogen, so it is safe to assume that the nitrogen isotope ratio of consumer tissues is mainly a function of that of the dietary protein sources, plus the trophic level enrichment effect described above. For nitrogen isotopes of consumer tissue proteins, you are what you eat plus 3–4‰ (Schoeninger and DeNiro, 1984; Minagawa and Wada, 1984). A 5‰ enrichment between diet and collagen $\delta^{13}C$ values has been observed in several studies of large mammals, including humans, on diets in which proteins and non-proteins have similar carbon isotope ratios (Vogel, 1978; Vogel and Van Der Merwe, 1978). For carbon isotopes in collagen, you are what you eat plus 5‰. Other tissues, including hair and flesh, have smaller degrees of enrichment, and fats tend to be depleted in ^{13}C relative to the diet (Vogel, 1978).

Diet reconstruction with carbon isotopes is usually based on the assumption that carbon atoms from all dietary macronutrient fractions (fats, carbohydrates and proteins) have an equal probability of being synthesized into consumer tissues. This is called the linear mixing model of diet-tissue isotopic relationships (Schwarcz, 1991). Most paleodietary studies have explicitly (Schoeninger, 1989) or implicitly assumed linear mixing for the relationship between diet and bone collagen carbon isotope ratios. However, Chisholm (1989; Chisholm et al., 1982) proposed that dietary protein is routed to tissue protein.

Which model is likely to be correct? The linear mixing model cannot be entirely correct because 12.2% of the amino acids in collagen come directly from essential amino acids that must be obtained from dietary protein sources (Klepinger and Mintel, 1983; Ambrose, 1993). The essential amino acids comprise 17.8% of the carbon atoms in collagen because the essential amino acids have more carbon atoms than the most abundant non-essential amino acid in collagen. An additional 1.5% of the carbon atoms in collagen come from non-essential amino acids (tryosine and hydroxylisine) that can only be synthesized from essential amino acid precursors (Rodwell, 1993a). This places a minimum estimate 19.3% of the amount of isotopic routing in collagen. Three of the most abundant non-essential amino acids in collagen (glycine, proline and hydroxyproline) are formed by transamination of other amino acids. These account for an additional 46.2% of the carbon atoms in collagen.

Since the synthesis of amino acids from non-amino acid precursors often incurs significant energetic costs it can be assumed that they will be preferentially obtained from dietary sources unless the diet provides quantities insufficient for tissue synthesis. Indeed, the essential amino acids require 5 to 10 enzymes for synthesis. Glycine, proline and hydroxyproline, which are the most abundant amino acids in collagen, each require four enzymes for neosynthesis. The remaining non-essential amino acids each require only one enzyme for neosynthesis (Rodwell, 1993a). If conservation of energy is adaptive then one would expect the amount of routing of carbon from dietary protein to tissue protein to be about 65% when the diet supplies an excess of each amino acid. The fact that humans turnover 1–2% of their total body protein daily and recycle approximately 75–80% of these amino acids for new protein synthesis (Rodwell, 1993b) is also consistent with preferential routing of protein carbon rather than predominant neogenesis of non-essential amino acids. Lowered efficiency of utilization of amino acids and their precursors may occur for a variety of reasons, including the presence of plant secondary compounds that inhibit digestion and absorption (Milton and Dintzis 1981) and the presence of poorly convertible amino acid stereoisomers produced by bacterial fermentation of milk products (Baker, 1994).

The absolute minimum amount of protein routing is theoretically only ~20%, but con-

sidering the facts outlined above, is undoubtedly much higher. This has an important implication for the use of stable carbon isotopes for diet reconstruction: where protein and non-protein resources have different carbon isotope ratios, collagen may not accurately reflect the isotopic composition of the whole diet.

No experiments or observations provide support for linear mixing or minimum routing models for collagen. However, experiments on the relationship between diet and bone collagen isotopic composition using rats and mice (Ambrose and Norr, 1993; Tieszen and Fagre, 1993) demonstrate that bone collagen carbon is indeed derived mainly from dietary proteins. The amount of routing greatly exceeds that predicted from the minimum amount that must be derived from essential amino acids. Our experiments with rats (Ambrose and Norr, 1993; unpublished data) will be summarized to illustrate the evidence for routing and the potential magnitude of errors in estimation of whole diet isotopic composition. These experiments, and those of Tieszen and Fagre (1993) have, however, revealed that bone apatite carbonate conforms to the linear mixing model. Until results of other controlled diet experiments become available we shall use the results of these experiments (Ambrose and Norr, 1993; unpublished data) to help reconstruct human diet in the Marianas.

Rats were raised from conception on diets with protein and non-protein (purified sugar, starch, fat and fiber) from opposite photosynthetic pathways (Ambrose and Norr, 1993). A second series of experiments used marine protein (tuna), combined with C_3 and/or C_4 terrestrial non-protein, and for some diets, tuna and terrestrial C_3 protein (unpublished data). Each diet configuration was prepared with 5%, 20% and 70% protein. Four to six offspring were raised on each diet. Male/female pairs were analyzed at 91, 131 and 171 (for litters of six) days after birth. The discussion below is based on the results on animals raised on 24 different configurations of protein amount and protein vs. non-protein carbon isotope composition.

Results from one pair of diet configurations clearly demonstrate how dietary protein predominantly labels tissue proteins (Ambrose and Norr, 1993). On a diet in which 94% of the carbon atoms came from C_3 carbohydrates and fats and 6% came from C_4 protein, and on the reverse diet, with 96% C_4 non-protein and 6% C_3 protein carbon, the whole diet $\delta^{13}C$ values differed by 12‰ but bone collagen values differed by only 1‰. The carbon isotope ratios of rat collagen on these diets suggests both diets had approximately 50% to 60% C_4, but one was 95% C_4 and the other was only 5% C_4 by weight. On these low-protein diets, approximately half the carbon atoms in collagen came from the protein alone. On the predominantly C_3 diet the collagen-diet difference ($\Delta^{13}C_{coll\text{-}diet}$) was +9.6‰ and on the C_4 diet it was −1.5‰. Mean $\Delta^{13}C_{coll\text{-}diet}$ values for our experiments range from −2.3‰ to +10.0‰ (a range of 12.3‰). When the $\delta^{13}C$ value of the protein source is more negative than that of the whole diet, a small $\Delta^{13}C_{coll\text{-}diet}$ is generated. Conversely, when the $\delta^{13}C$ value of the protein source is less negative than that of the whole diet, a large $\Delta^{13}C_{coll\text{-}diet}$ results. Although these rats were not what they ate plus 5‰, the diet-collagen difference was a simple linear function of the difference between the $\delta^{13}C$ value of the whole diet and that of the dietary protein. These experiments demonstrate that the diet-collagen function is variable, but it can be explained by the difference between the $\delta^{13}C$ value of the protein source and the whole diet.

These experiments have also confirmed that the diet-collagen difference value is approximately 5‰, but *only* when protein and non-protein diet components have identical $\delta^{13}C$ values. This enrichment factor was insensitive to the amount of protein in the diet. In other words, when $\Delta^{13}C_{prot\text{-}diet} = 0$‰, rats were what they ate plus about 5‰ on low, normal and high protein diets.

If our rat experiments are applicable to humans, the results suggest that when the $\delta^{13}C$ values of low- and high-protein foods differ, the contribution of low protein foods to diets may be underestimated by carbon isotope analysis of bone collagen. This situation is often found in human diets. For example, animal foods from Micronesia average 84% protein by weight, but plant foods average only 5% protein. The starchy staples

have even less protein. The $\delta^{13}C$ values of terrestrial plants and marine animals in this study differ by almost 13‰. Terrestrial plant resource use in Micronesia will probably be underestimated by bone collagen $\delta^{13}C$ values.

Diet-apatite carbonate relationships

Bone apatite carbonate is derived from serum CO_2 and bicarbonate, which is generated by energy metabolism. Controlled diet experiments with rodents demonstrate that the carbon isotopic composition of bone apatite carbonate accurately reflects that of the whole diet. It does not over- or underestimate the proportions of carbon atoms from fats, carbohydrates and proteins (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). Both experimental programs found a remarkably simple and consistent result for the $\Delta^{13}C_{carb\text{-}diet}$: you are *always* what you eat plus about 9.4‰. In our experiments, the $\Delta^{13}C_{carb\text{-}diet}$ could only be forced to vary between +8.8‰ and +10.9‰ (a range of only 2.1‰) regardless of the proportions of proteins to non-proteins or the magnitude of difference of their carbon isotopic compositions. The linear mixing model is appropriate for bone carbonate under an extremely wide range of dietary configurations. These experiments demonstrate that virtually all dietary macronutrients are ultimately converted to energy. Therefore whole diet carbon isotopic composition can be reconstructed from analysis of bone carbonate.

Diet reconstruction with bone collagen and carbonate carbon isotopes

The results of controlled diet experiments have supported aspects of models of diet reconstruction with stable carbon isotope ratios of bone collagen and bone carbonate proposed by Krueger and Sullivan (1984) and Lee-Thorp et al. (1989), but the values for diet-tissue functions obtained in our experiments differed from their estimates based on analyses of free-ranging animals. Krueger and Sullivan (1984) suggested no enrichment in $\delta^{13}C$ values between dietary protein and collagen (ie. direct routing without isotopic fractionation), and a large enrichment between non-protein and collagen. Controlled diet experiments show enrichment is always +5‰ on diets with 5%, 20% and 70% protein, but only when all dietary macronutrients have the same $\delta^{13}C$ values.

Bone carbonate $\delta^{13}C$ values have been considered to be an indicator of trophic level because herbivores and carnivores have estimated $\Delta^{13}C_{carb\text{-}diet}$ values of 11–14‰ and 8–9‰, respectively (Krueger and Sullivan, 1984; Lee-Thorp, 1989). The most widely accepted explanation for the difference in carbonate-diet spacing between herbivores and carnivores is based on the assumption that carbonate carbon is derived from energy metabolism, which is confirmed by our controlled diet experiments. Energy is assumed to be mainly carbohydrates and fats (Lee-Thorp et al., 1989), plus protein that is not used for tissue synthesis (Krueger and Sullivan, 1984). As noted above, fats have lower $\delta^{13}C$ values than other dietary macronutrients. If carnivores derive a greater proportion of energy from fats, then they should have lower carbonate $\delta^{13}C$ values. Our experiments (Ambrose and Norr, 1993) were not designed to test the effect of varying the isotopic composition of fats on carbonate $\delta^{13}C$ values, but Tieszen and Fagre (1993) included one diet in which fat $\delta^{13}C$ values were changed in a way that should have affected diet-carbonate difference values. The $\Delta^{13}C_{carb\text{-}diet}$ value on this diet was exactly 9.4‰. This experiment raises significant questions about the role of fats in the currently favored trophic level model of carbonate-diet spacings.

An alternative explanation for the difference in $\Delta^{13}C_{carb\text{-}diet}$ values between herbivores and carnivores, proposed by Hedges and Van Klinken (in press), is based on the observation that ruminant herbivores support a microbial community that generates a substantial amount of methane. In-vitro and in-vivo studies of ruminant herbivore methanogenesis (Metges et al., 1990) show that ruminant methane has a very negative $\delta^{13}C$ value relative to the diet. Low methane $\delta^{13}C$ values are balanced by respired CO_2 with very enriched $\delta^{13}C$ values. Hedges & Van Klinken (in press) propose that this enriched CO_2 explains high herbivore $\Delta^{13}C_{carb\text{-}diet}$ values. Since rats, humans and carnivores produce relatively small amounts of methane compared to ruminants, they prob-

ably do not achieve such high $\Delta^{13}C_{carb\text{-}diet}$ values simply by eating low protein/low fat diets. The unusual isotopic features of ruminant herbivore digestion may make them an inappropriate model for reconstructing the diets of other animals. Therefore the appropriate $\Delta^{13}C_{carb\text{-}diet}$ value for humans is probably ~9.4‰ rather than 12–13‰.

The results of our controlled diet experiments show that when both carbonate and collagen carbon isotope ratios of the same individual are analyzed, the carbon isotopic composition of protein and non-protein components of prehistoric diets can be reconstructed. When protein and non-protein components of diets have similar $\delta^{13}C$ values (when $\Delta^{13}C_{prot\text{-}diet} = 0‰$) the collagen-diet difference is +5‰. The $\Delta^{13}C_{carb\text{-}diet}$ is always ~9.4‰, so on a mono-isotopic diet the carbonate-collagen difference ($\Delta^{13}C_{carb\text{-}coll}$) is +4.4‰. The whole diet $\delta^{13}C$ value is estimated by subtracting 9.4‰ from the carbonate $\delta^{13}C$ value. The $\delta^{13}C$ value of dietary protein can then be estimated from the $\Delta^{13}C_{carb\text{-}coll}$ value. When $\Delta^{13}C_{carb\text{-}coll}$ is greater than 4.4‰, then the $\delta^{13}C$ value of dietary protein is more negative than that of the whole diet. A diet of predominantly C_4 carbohydrates and C_3 proteins would produce this pattern. When the difference is less than 4.4‰, dietary protein is less negative than whole diet. This would be consistent with a diet comprising mainly C_3 carbohydrates plus marine proteins.

MATERIALS AND METHODS

Skeletal materials

Human skeletal remains were obtained during the course of archaeological excavations on Rota, Guam and Saipan islands, directed by Butler (1988, 1995), Hunter-Anderson (1994; Amesbury et al., 1991) and the Historic Preservation Office of the Commonwealth of the Northern Marianas, respectively. Hanson (1988, 1989, 1991, 1995) analyzed human skeletal biology and pathology. Isotopic analyses of bone collagen and apatite carbonate have been performed by Ambrose at the University of Illinois and Krueger at Geochron Laboratories.

The burials from Rota were recovered from four village sites on the north coast. All but one post-date 1000 A.D. (Butler, 1988). Several were associated with the remnants of Latte structures. The skeletal remains from the Duty-Free site on Saipan are directly dated to the Latte Period (1250–1350 A.D.). The San Antonio Burial Trench site on Guam is also assigned to the Latte Period on the basis of burial style and radiocarbon dates ranging from 950 to 1350 AD (Amesbury et al., 1991). Most human burials from Nansay and all from MacHomes on Saipan also date to the Latte phase (Butler, 1995). One well-preserved burial from Nansay (Nansay 4) was associated with a brass flushloop bell and probably dates to between 1668 and 1730 A.D. (Butler, 1995:77). This individual may have lived during a period of violent clashes with the Spanish between 1670 and 1695 A.D. (Butler 1995).

Dietary resources

The stable carbon and nitrogen isotopic composition of a representative sample of dietary items collected by Hunter-Anderson from Guam, Yap and Tobi (Palau) has been determined in order to increase the accuracy of our reconstruction of prehistoric Marianas diets (Table 1). Some staple foods were analyzed in raw and traditionally processed states. For example, breadfruit was analyzed (1) raw, (2) fermented, (3) boiled and (4) fermented, wrapped in leaves and baked in an earth oven; cycad seed flour after leaching; bananas, yams, sweet potatoes, turmeric and chili peppers both raw and boiled. Animal flesh rather than bone or shell was analyzed. One sample of uncooked edible seaweed from the Marianas has also been analyzed.

Sample preparation for isotopic analysis

Bone collagen and apatite carbonate were prepared at the Illinois lab by procedures described in detail elsewhere (Ambrose 1990, 1993). Collagen was prepared by demineralization of ground bone (0.25–0.5 mm sieved fraction) in either 1 M HCl for 20 minutes (for bones from Rota) or 0.2 M HCl (Guam and Saipan) for 1–3 days, followed by treatment with 0.125 M NaOH for 20 hours to remove soil humic acids, solubilization (gelatinization) by heating in weak acid (pH 3) at 95°C for 10 hours, filtration through a glass frit filter and freeze-drying. The gelatin resi-

due is predominantly collagen but may contain small amounts of non-collagenous proteins, salts and other biochemical fractions that are not removed by acid, base or filtration treatments. Freeze-dried collagen (8–12 mg) was placed in Vycor glass tubes with Cu, CuO and Ag foil, evacuated, sealed and combusted in a muffle furnace at 860°C for three hours and cooled slowly for 15 hours. Combustion converted organic matter to CO_2, N_2 and water, which were separated by cryogenic distillation. N_2 was collected with a toepler pump to avoid nitrogen isotope fractionation during distillation and collection.

Apatite carbonate was purified by treatment of finely ground bone (<0.25 mm sieved fraction) with 50% Clorox for 1–3 days to remove organic matter, followed by 1 M acetic acid for 20–40 hours to remove diagenetic and adsorbed carbonates. Structural carbonate (Lee-Thorp and Van Der Merwe, 1991) was converted to CO_2 by reaction with 100% phosphoric acid at 25°C and collected by cryogenic distillation.

Modern plant and animal food samples were prepared by grinding to <1.0 mm with a Wiley mill, followed by sealed tube combustion, cryogenic distillation and mass spectrometry. The seaweed sample was split and one aliquot was treated with 1 M HCl to remove carbonates.

Atomic C:N ratios and weight percent N and/or C in collagen, apatite and food samples were determined during cryogenic distillation by manometric measurement of the volumes of gases generated by combustion or acid reaction. These data are used to assess collagen and apatite preservation, contamination (Ambrose, 1990, 1993), food bulk elemental composition and protein content. Mass spectrometry was performed at the Illinois State Geological Survey by Jack Liu, except for nitrogen isotope ratios for Rota, which were determined at the University of Illinois Department of Agronomy by Richard Mulvaney.

Collagen and bioapatite (Krueger, 1991) sample preparation at Geochron Laboratory involved pretreatment of ground bone by reaction with 1 M acetic acid under weak vacuum to remove adsorbed carbonates. Bioapatite carbonate was reacted with HCl to release CO_2. The demineralized collagen residue was then gelatinized, dried, and prepared for sealed tube combustion, cryogenic distillation and mass spectrometry, as described above. Overall precision of isotopic analyses in both labs is ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

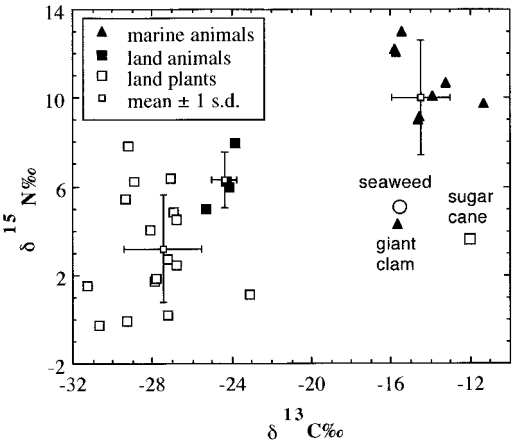

Fig. 1. Stable carbon and nitrogen isotope composition of dietary resources listed in Table 1. Bivariate means and standard deviations for major categories of food resources are also plotted.

RESULTS

Marianas foodweb stable isotopes

Table 1 lists the carbon and nitrogen concentrations and isotopic composition of dietary items. Many of the starchy plant foods produced inadequate nitrogen for isotopic analysis. The bivariate means and standard deviations for marine and terrestrial plants and animals, as well as the individual values of samples for which both carbon and nitrogen isotope ratios were determined, are shown in Figure 1. As noted above, plant foods have much lower nitrogen contents than animal flesh. The protein content of animal matter can be estimated by multiplying the weight %N by 6.25. The average nitrogen content of animal foods analyzed in this study is ~13.5% by weight, which is equivalent to ~85% protein. The average nitrogen content of plant foods analyzed in this study is ~0.8%, which should be equivalent to about 5% protein. However, Milton and Dintzis (1981) suggest a more appropri-

TABLE 2. Organic and inorganic chemical and isotopic composition of human bones from sites on Rota Island

			Collagen[1]							Apatite carbonate[1]				
Site	Sex, Age	Feature, Burial #	Lab # (NS)	Collagen wt.%	C wt.%	N wt.%	C:N	$\delta^{15}N$‰	$\delta^{13}C$‰	Lab # (CH)	Apatite wt.%	C wt.%	$\delta^{13}C$‰	$\Delta^{13}C‰_{carb\text{-}coll}$
Teteto-Guata	M?, adult	F11, B1	14	3.4	28.0	9.5	3.7	9.3	−17.9	87	38.1	1.12	−11.0	6.9
Teteto-Guata	F? adult	F40, B6	5	6.8	38.1	13.4	3.3	7.6	−19.4	79	35.9	1.07	−12.4	7.0
Teteto-Guata	M?, 15–17	F43, B8	8	3.2	27.9	9.5	3.4	7.3	−19.2	81	41.7	1.12	−11.1	8.1
Teteto-Guata	F, 45–50	F49, B9	9	5.5	36.1	12.5	3.4	10.9	−17.4	82	24.1	0.83	−12.7	4.7
Teteto-Guata	M?, adult	F50, B11	10	2.9	23.6	7.9	3.5	8.3	−18.0	83	36.5	1.22	−11.1	6.9
Unginao-Uyulan	?, adult	F62, B15	11	4.2	26.2	8.9	3.4	7.8	−19.0	84	38.2	0.93	−11.1	7.9
Unginao-Uyulan	M?, 16–19	F66, B19	12	4.9	24.1	10.0	3.4	9.3	−17.4	85	37.3	1.16	−10.1	7.3
Unginao-Uyulan	?, adult	F73, B22	13	3.9	25.1	8.7	3.4	11.3	−15.7	86	40.3	1.15	−10.2	5.5
Salug-Songton	F, 35–39	F38, B5	15	6.8	36.8	12.9	3.3	8.8	−18.2	88	36.0	0.95	−12.9	5.3
Salug-Songton	F, 30–40	F37, B4	6	6.2	38.4	13.5	3.3	9.4	−18.3	80	17.4	1.00	−12.7	5.6

[1] Explanation of column headings: Lab sample number (notebook prefix) collagen concentration in whole bone, weight % C and N in collagen, atomic C:N ratio, N and C isotopic composition; lab sample number (notebook prefix), apatite concentration in whole bone, carbonate C concentration in apatite, C isotope composition, and difference (Δ) between carbonate and collagen $\delta^{13}C$ values.

ate nitrogen-to-protein conversion factor for leaves is 4.4 because a substantial proportion of nitrogen is contained in indigestible and even toxic forms such as alkaloids, cyanogenic glycosides and other secondary compounds. The conversion factor for seeds and starches may also be lower than 6.25 because antagonistic interactions with fiber, phytate, trypsin inhibitors, Maillard reaction products from cooking and other chemical reactions can lower amino acid bioavailability and protein quality (Sarwar, 1997). The proportion of digestible N in plant foods used in the Marianas is probably highest for fruits and lowest for cycad seeds, which have high levels of neurotoxic glycosides and nonprotein amino acids (Spencer et al., 1987), and some varieties of tubers and chilies. The digestibility of seaweeds is uncertain but may be high, as they have comparatively small amounts of cellulose and lignin. Food preparation may also affect nitrogen concentrations. Boiled plant food samples had lower nitrogen contents than raw ones for seven of eleven species that were analyzed both boiled and raw (Table 1).

Terrestrial plants analyzed in this study are all C_3, with a mean $\delta^{13}C$ value of −27.4‰ (Table 1). Rice was not analyzed in this study, but $\delta^{13}C$ values for purified rice starch used in our controlled diet experiments (Ambrose and Norr, 1993) averaged −26.6‰. The only traditional prehistoric C_4 food plant known in the Marianas is sugar cane, which was not analyzed in this study. The mean $\delta^{13}C$ value for purified cane sugar in our experimental diets is −11.2‰. The terrestrial animal flesh mean is −24.4‰, and that for marine animals is −14.5‰. One species of edible seaweed (red algae) from Guam has a $\delta^{13}C$ value of −15.6‰.

Domesticated crops from the Marianas have a mean $\delta^{15}N$ value of +3.2‰, and that for terrestrial animals is +6.3‰. Land animals analyzed include fruit bat, land crab and coconut crab. The $\delta^{15}N$ value for the edible seaweed from Guam is +5.0‰. The mean $\delta^{15}N$ value for the flesh of nine marine animal species from Guam is +10‰. The filter-feeding tridacnid clam has a $\delta^{15}N$ value of only +4.3‰. Deep-water carnivorous fish have $\delta^{15}N$ values over +12‰. Reef and lagoon fish and spiny lobsters have intermediate $\delta^{15}N$ values, of around +10‰. The foodweb carbon and nitrogen isotope values we obtained in Micronesia are similar to those from Caribbean islands compiled by Keegan and DeNiro (1988).

Human bone preservation and stable isotope composition

The organic and inorganic elemental and isotopic composition of human bone collagen and apatite carbonate are shown in Tables 2–4 and Figures 2–4. Descriptive statistics on stable isotopic composition, grouped by island, are presented in Table 5. Bivariate means and standard deviations are plotted in figures 2d, 3d and 4d.

Ten individuals from Rota have been analyzed (Table 2). Bone collagen concentrations are relatively low (4.8 ± 4%), which is typical for prehistoric bones from warm tropical environments. Fresh bone is about 20% collagen. The atomic C:N ratios of collagen are all within the acceptable range of 2.9 to

TABLE 3. Organic and inorganic chemical and isotopic composition of human bones from Backhoe Trench 1[1] and San Antonio Burial Trench[2,3] Agana, Guam

			Collagen[4]							Apatite carbonate[4]				
Site	Sex, Age	Burial No.	Lab # (HC)	Collagen wt.%	C wt.%	N wt.%	C:N	$\delta^{15}N‰$	$\delta^{13}C‰$	Lab # (CH)	Apatite wt.%	C wt.%	$\delta^{13}C‰$	$\Delta^{13}C‰_{carb\text{-}coll}$
Backhoe Trench	?	1	33	4.8	40.6	14.8	3.2	9.3	−17.4	128	29.3	1.36	−11.6	5.8
SABT[2]	M, 23+	2						9.0	−17.9				−12.3	5.6
SABT	F, 25–30	3						9.8	−16.7				−12.5	4.2
SABT	F, 35+	5						9.1	−18.1				−12.1	6.0
SABT	F, 25+	6						10.1	−16.8				−12.2	4.6

[1] Analyzed by Ambrose.
[2] San Antonio Burial Trench.
[3] Analyzed by Krueger.
[4] See Table 2 legend for explanation of column headings.

TABLE 4. Organic and inorganic chemical and isotopic composition of human bones from MacHomes and Nansay sites, Achugao,[1] and the Duty Free Site, Garapen Village, Saipan Island[2]

			Collagen[3]							Apatite carbonate[3]				
Site	Sex, Age	Burial No.	Lab # (HC)	Collagen wt.%	C wt.%	N wt.%	C:N	$\delta^{15}N‰$	$\delta^{13}C‰$	Lab # (CH)	Apatite wt.%	C wt.%	$\delta^{13}C‰$	$\Delta^{13}C‰_{carb\text{-}coll}$
MacHomes	F, young adult	2	29	5.0	30.4	11.0	3.2	8.4	−18.0	124	43.1	1.24	−10.2	7.8
MacHomes	?, adult	5	30	5.4	31.8	11.8	3.1	8.4	−18.3	125	45.1	1.20	−7.7	10.6
Nansay	?, 14–17	7	31	5.5	39.8	14.7	3.2	8.1	−18.6	126	38.1	1.13	−8.7	9.9
Nansay	F, 50+	6	32	6.9	41.1	15.2	3.2	7.9	−19.0	127	39.6	1.11	−9.7	9.4
Nansay	F, 40–44	4	43	9.7	37.8	14.1	3.1	9.2	−16.6	138	37.9	1.18	−12.3	4.3
Duty Free Site	F, old adult	1						7.1	−18.6				−9.6	9.0
Duty Free Site	M, old adult	2						6.1	−18.9				−8.5	10.4
Duty Free Site	F, 39–45	3						7.5	−18.7				−9.1	9.6
Duty Free Site	M, 20–22	4						9.2	−18.5				−10.0	8.5

[1] Analyzed by Ambrose.
[2] Analyzed by Krueger.
[3] See Table 2 legend for explanation of column headings.

3.6 for well-preserved samples (DeNiro, 1985), as are collagen C and N concentrations (Ambrose, 1990).

Apatite concentrations (weight %) reflect the amount of inorganic bone remaining after removal of organic matter and acid-soluble inorganic phases, plus physical losses of ultrafine fractions during centrifugation and decantation. Higher apparent apatite yields were found in bones that had less organic matter before pretreatment because they initially had proportionately less organic matter.

Apatite carbonate carbon concentrations for modern African mammals average 0.90 ± 0.14% (Ambrose, unpublished data). Those from Rota average 1.06 ± 0.12%, which is above the range of modern bone. This 0.16%

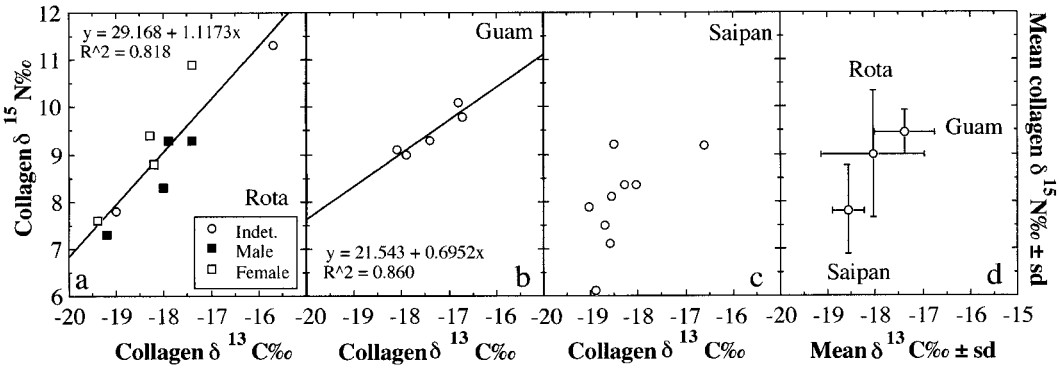

Fig. 2. Bone collagen stable carbon and nitrogen isotope values for humans from Rota Island (a), Guam (b), Saipan (c) and bivariate means and standard deviations for each island (d).

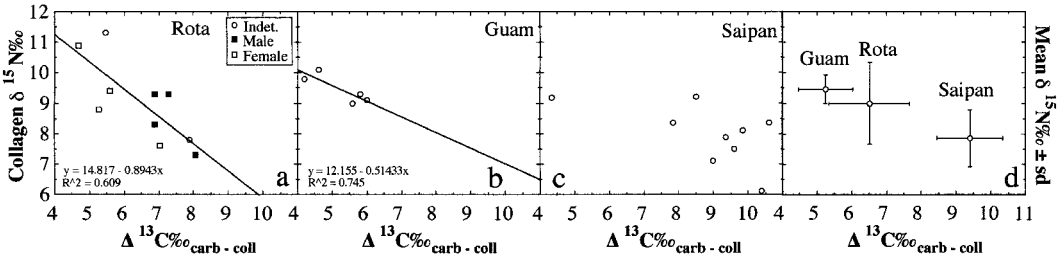

Fig. 3. Bone apatite-collagen carbon isotope difference ($\Delta^{13}C_{carb\text{-}coll}$) and collagen nitrogen isotope values for humans from Rota Island (a), Guam (b), Saipan (c) and bivariate means and standard deviations for each island (d).

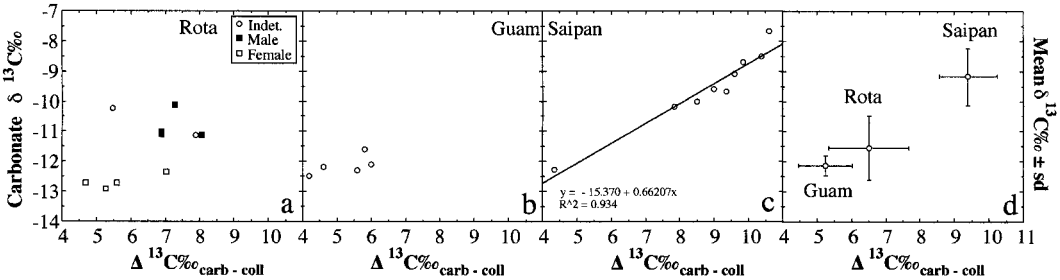

Fig. 4. Bone apatite-collagen carbon isotope difference and carbonate carbon isotope values for humans from Rota Island (a), Guam (b), Saipan (c) and bivariate means and standard deviations for each island (d).

average excess of carbonate carbon could be explained in three ways. First, modern bone retains a significant amount of organic matter after treatment with Clorox (DeNiro and Weiner, 1988). The prehistoric bones had very low collagen contents before pretreatment and deproteinization could have removed proportionately more collagen, leaving proportionately more biological carbonate. Second, high carbonate yields could result from incomplete removal of diagenetic carbonate adsorbed on apatite crystal surface positions. Third, if apatite crystallinity increased, this slight excess could reflect either incorporation of diagenetic carbonate in structural positions or the retention of biological structural carbonate in more acid-resistant bone mineral. X-ray diffraction analysis could be used to evaluate crystallinity changes, but was not performed. If diagenetic carbonate has been incorporated then one would expect a correlation between carbonate %C and $\delta^{13}C$, but none was found.

Collagen $\delta^{13}C$ values on Rota range from −19.5‰ to −15.7‰, and are strongly correlated with $\delta^{15}N$ values (Fig. 2a), as would be expected for people whose diets comprised varying proportions of deep water marine versus terrestrial C_3 resources. High $\delta^{15}N$ values for some individuals suggest con-

TABLE 5. Descriptive statistics for the isotopic composition of bone collagen and apatite carbonate of prehistoric humans from Rota, Guam and Saipan

	Collagen $\delta^{15}N$‰				Collagen $\delta^{13}C$‰				Carbonate $\delta^{13}C$‰				$\Delta^{13}C_{carb\text{-}coll}$‰				
Island	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	N
Rota	9.00	1.34	7.3	11.3	−18.05	1.08	−19.4	−15.7	−11.55	1.05	−12.9	−10.1	6.51	1.17	4.7	8.1	10
Guam	9.46	0.47	9.0	10.1	−17.38	0.63	−18.1	−16.7	−12.14	0.34	−12.5	−11.6	5.24	0.79	4.2	6.0	5
Saipan[1]	7.82	0.94	6.1	9.2	−18.58	0.32	−19.0	−18.0	−9.18	0.86	−10.2	−7.7	9.40	0.94	7.8	10.6	8

[1] Burial Nansay 4 has been excluded from the statistics for Saipan because it is a historic-period individual who may have emigrated from Guam.

sumption of large amounts of deep water fish, which is consistent with archaeological faunal evidence on Rota. Low collagen $\delta^{13}C$ and $\delta^{15}N$ values for others suggest predominantly terrestrial diets. Apatite carbonate $\delta^{13}C$ values range from $-12.9‰$ to $-10.1‰$ and are very poorly correlated with collagen $\delta^{15}N$ and $\delta^{13}C$ values. These poor correlations may indicate that the protein and non-protein dietary resources have different carbon isotopic compositions. The mean difference between apatite and collagen $\delta^{13}C$ is 6.5‰, which indicates the protein $\delta^{13}C$ values was more negative than that of the whole diet. Low collagen $\delta^{13}C$ values indicate this was mainly from terrestrial sources, which could include plant foods, fruit bats and land crabs. The $\Delta^{13}C_{carb\text{-}coll}$ decreases with increasing $\delta^{15}N$ (Fig. 3a), which indicates the protein carbon in the diet was predominantly marine for individuals with high $\delta^{15}N$ values.

Females tend to have slightly higher $\delta^{15}N$ values for their collagen $\delta^{13}C$ values than males (Fig. 2a). Males tend to have higher $\Delta^{13}C_{carb\text{-}coll}$ values because they have higher carbonate $\delta^{13}C$ values (Fig. 4a), but this gender difference may not be significant if the unsexed individuals are female. Sample sizes for each sex are small so the differences in isotopic composition may not be statistically significant.

The isotopic composition of human bones from Guam is similar to that from Rota. There is a strong positive correlation between collagen $\delta^{13}C$ and $\delta^{15}N$ values, but $\delta^{15}N$ values are somewhat lower than those from Rota (Fig. 2b). They also have small $\Delta^{13}C_{carb\text{-}coll}$ values (Fig. 3b) and lower carbonate $\delta^{13}C$ values (Fig. 4b). These data suggest diets with mainly terrestrial C_3 resources combined with marine protein, and less reliance on seaweeds and/or C_4 plants. Two individuals with $\Delta^{13}C_{carb\text{-}coll}$ values of slightly less than 4.4‰ apparently ate virtually no ^{13}C-enriched plant foods. Their high $\delta^{15}N$ values demonstrate greater reliance on marine protein.

Carbon isotopes of apatite and carbonate on Rota and Guam provide discordant evidence for diets. Carbon and nitrogen isotopes of collagen suggest the diets comprised varying proportions of marine versus mainly terrestrial C_3 resources. Carbonate carbon isotopes suggest the presence of resources with high $\delta^{13}C$ values but low protein contents. The strong correlation between collagen $\delta^{15}N$ and $\delta^{13}C$ values and low correlation between collagen and carbonate $\delta^{13}C$ values is consistent with the protein routing model for collagen and linear mixing model for carbonate.

Individuals from the Saipan sample set have the lowest collagen $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2c), but they have unusually high carbonate $\delta^{13}C$ values and thus $\Delta^{13}C_{carb\text{-}coll}$ values as high as 10.6‰ (Figs. 3c and 4c). The combination of high carbonate $\delta^{13}C$ values with low collagen $\delta^{13}C$ and $\delta^{15}N$ values for all individuals except for Nansay burial 4, indicates they ate significant amounts of protein from C_3 sources and consumed small amounts of marine foods, perhaps reef and lagoon fish and shellfish, which have low $\delta^{15}N$ values. This is consistent with the larger lagoon and reef habitats on Saipan. However, very high carbonate $\delta^{13}C$ and $\Delta^{13}C_{carb\text{-}coll}$ values demonstrate C_4 carbohydrates and/or seaweeds must have been significant dietary resources. The isotopic composition of Nansay 4 is indistinguishable from that of individuals from Guam.

DISCUSSION

The diets of individuals from Rota and Guam are fairly similar, but most individuals from Saipan had a significantly different diet. Although low mean collagen $\delta^{15}N$ values on Saipan hint at this difference (Fig. 2d), its cause is only clearly revealed by analysis of apatite carbonate $\delta^{13}C$ (Figs. 3d and 4d). Substantial amounts of plant foods with high $\delta^{13}C$ values and very low protein contents must have been consumed on Saipan. Smaller amounts of such resources were consumed on Rota and even less were consumed on Guam. Saipan collagen $\delta^{13}C$ values indicate the lowest proportions of marine and/or C_4 plants, but this clearly misrepresents the carbon isotopic composition of the whole diet as reflected in carbonate $\delta^{13}C$ values.

The mean $\delta^{13}C$ values for marine and terrestrial dietary resources of the local foodweb can be used to determine the per-

TABLE 6. Estimates of percentage of marine resources in the diet of human populations from the Marianas based on descriptive statistics of carbon isotopic composition of bone collagen and carbonate listed in Table 5

	% Marine from Collagen				% Marine from Carbonate				
Island	Mean	SD	Min	Max	Mean	SD	Min	Max	$\Delta M_{carb\text{-}coll}$‰[1]
Rota	21.3	9.4	9.6	41.8	39.6	9.1	27.8	52.2	18.3
Guam	27.1	5.5	20.9	33.1	34.5	3.0	31.3	39.2	7.3
Saipan	16.7	2.8	13.1	21.8	60.2	7.5	51.3	73.1	43.5
Saipan C_4[2]	16.7	2.8	13.1	21.8	47.7	5.9	40.7	58.0	31.0

[1] Difference between estimate of % marine in diet from carbonate and collagen $\delta^{13}C$ values.
[2] Calculated using assumptions of C_4 end-member with no protein. If the diet-to-tissue routing of protein model is correct this will have little effect on collagen $\delta^{13}C$ values.
See text for definition of isotopic end-members for terrestrial and marine resources.

centage of marine foods in the diets of individuals on each island. Based on the data in Table 1, the foodweb end-member $\delta^{13}C$ values for marine and terrestrial C_3 resources are −14.5 and −27.1‰, respectively. Correcting the means for the industrial effect on air CO_2 $\delta^{13}C$ values would add ~1.6‰ to the mean for terrestrial C_3 (−25.5‰) and ~0.5‰ (−14.0‰) to that for marine resources. The difference between marine and terrestrial C_3 end-members is about 11.5‰, so each 1‰ difference in $\delta^{13}C$ away from one end-member equals an 8.7% change toward the opposite end-member. The uncorrected mean $\delta^{13}C$ value for terrestrial animal flesh is −24.4‰. High-protein animal foods would have contributed proportionately more carbon to collagen than the low-protein starchy plant staples. The pure C_3 end-member value is thus probably too negative, but how much the mean should be weighted toward dietary protein cannot be estimated accurately without knowledge of the proportions of animal versus plant foods consumed and the degree of routing of dietary protein to collagen. The most appropriate value for the C_3 end-member thus depends upon assumptions about the proportions of carbon derived from protein versus non-protein and proportions of plant versus animal protein in prehistoric diets.

Using the modern unweighted mean for terrestrial C_3 plant plus animal resources, corrected for the industrial effect (−25.5‰), and the collagen-diet difference of +5‰, produces a collagen $\delta^{13}C$ value of −20.5‰ for a pure C_3 prehistoric diet on the Marianas. Using the mean for marine resources, corrected for the industrial effect (−14.0‰), the prehistoric human collagen $\delta^{13}C$ value for a pure marine diet would be −9‰. Assuming carbonate $\delta^{13}C$ = whole diet plus 9.4‰, the pure C_3 and marine end-members for bone carbonate $\delta^{13}C$ values on the Marianas would be −16.1‰ and −4.6‰, respectively.

If C_4 plants with very low protein contents were also a significant dietary resource then the marine end-member for carbonate carbon isotopes would have to be adjusted to a less negative value. If this was a simple terrestrial ecosystem with no marine resources then the C_4 plant end-member $\delta^{13}C$ value (adjusted for the industrial effect) would be approximately −11‰ and the carbonate value would be about −1.6‰. In this case the C_3 and C_4 end-members would differ by 14.5‰ and each 1‰ difference from one end-member would reflect a 6.9% change toward the other.

Estimates of percent marine foods in the diet of each island's population based upon the assumptions for marine and terrestrial end-member values and diet-tissue functions are summarized in Table 6. If C_4 plant resources were also exploited then the carbonate values may overestimate marine resource consumption. For Saipan, an alternative calculation for % C_4 from carbonate using a pure C_4 end-member is included to illustrate how the assumed end-member values affect diet composition estimates.

On Rota, collagen $\delta^{13}C$ values suggest that marine foods averaged about 21 ± 9% of the diet and ranged from about 10% to 42%. Assuming no C_4 plants were used, carbonate $\delta^{13}C$ values indicate the average diet on Rota was 40 ± 9% marine, ranging from 28% to 52% marine. The difference between estimates of marine resource use from collagen and carbonate $\delta^{13}C$ values averages 18%, but ranges from 3% to 31%. This difference in estimates suggests some individuals con-

sumed significant amounts of a low-protein resource with high $\delta^{13}C$ and low $\delta^{15}N$ values. This could be a species of seaweed with higher $\delta^{13}C$ values than the one species analyzed in this study, and/or a C_4 plant. The only known economically significant C_4 plant in the Pacific is sugar cane.

On Guam there are no significant discrepancies between diet reconstructions based on collagen versus carbonate carbon isotopes. Both suggest the five individuals consumed about 27–35% marine resources. The mean $\Delta^{13}C_{carb\text{-}coll}$ of 5.24‰ (Table 5) indicates the non-protein component of the diet has a slightly higher $\delta^{13}C$ value than the protein source. This may reflect consumption of small amounts of seaweed and/or sugar cane.

The discrepancies in diet reconstruction based on collagen versus carbonate are greatest for Saipan. Collagen $\delta^{13}C$ values suggest marine protein comprised 17 ± 3% of the diet (range = 13–22%), while apatite carbonate $\delta^{13}C$ values suggest 60 ± 8% (51–70%) marine foods. The difference in estimates of % marine food use averages 44%. The mean $\Delta^{13}C_{carb\text{-}coll}$ is 9.0 ± 2‰ (Table 5), which is twice the value expected when the protein and non-protein diet components have the same carbon isotope ratios. If the high ^{13}C resource had significant amounts of protein, the $\Delta^{13}C_{carb\text{-}coll}$ values would be approximately 4.4‰ so this resource must have virtually no protein. Saipan carbonate $\delta^{13}C$ values show the whole diet $\delta^{13}C$ value was much higher than that of the diet's protein. Low $\delta^{15}N$ values for this population support the conclusion that the high $\delta^{13}C$ values of carbonate cannot be due to consumption of marine animal resources. The alternative calculation assuming a pure C_4 end-member (Table 6) suggests a smaller discrepancy (31% versus 44%) between collagen and carbonate estimates of marine food consumption.

A low $\Delta^{13}C_{carb\text{-}coll}$ value was obtained for only one of the nine individuals from Saipan (Table 4). This individual apparently dates to a period of violent clashes with the Spanish that occurred between 1670 and 1695 AD (Butler 1995). Many Chamorro fled Guam and resettled on Saipan and other northern islands. The isotopic composition of Nansay Burial 4 is indeed closely similar to those on Guam. It is also possible that this individual lived most of her life on Saipan, but the post-colonial diet of Saipan may have been similar to that observed on Guam. As noted above, there are few records of lifeways on Saipan during the first century of contact, but the written records of diet during historic period diet on Guam (Pollock, 1986) are consistent with the isotopic composition of Nansay 4 and the sample sets from Guam and Rota.

Our results from Saipan can be compared to those on bone collagen from the Latte Period site of Afetna, southwest Saipan (McGovern-Wilson and Quinn, 1996). Apatite carbonate was not analyzed in their study. The sample preparation methods are quite different from almost all previous studies (Ambrose, 1993), so the accuracy and precision of their results are uncertain. Nitrogen isotope ratios were determined on whole bone rather than purified collagen. Nitrogen was distilled by the Kjeldahl method, which has a lower accuracy and precision than sealed tube combusion (Minagawa et al., 1984). The mean bone collagen $\delta^{13}C$ and $\delta^{15}N$ values are −18.7 ± 0.8‰ and +9.5 ± 0.9‰, respectively. Their mean $\delta^{13}C$ value is not significantly different from that obtained in our study, but our mean $\delta^{15}N$ value is almost 1.5‰ lower (Table 5), which may indicate a diet with lower proportions of marine protein. We found a very poor correlation ($R^2 = 0.383$) between $\delta^{13}C$ and $\delta^{15}N$ values in our sample set from Saipan (Fig. 2c) and there is no correlation ($R^2 = 0.040$) for Afetna. In contrast, the correlation is highly significant on Rota ($R^2 = 0.810$, $P < 0.001$) and significant on Guam ($R^2 = 0.860$, $P = 0.023$) despite the small sample size (n = 5). The lack of correlation between carbon and nitrogen isotope ratios for Afetna (n = 10) is notable because it suggests that the diet includes resources with high $\delta^{13}C$ but low $\delta^{15}N$ values. Apatite carbon isotopes demonstrated this was the case in our sample set and it may also explain the Afetna data.

The high apatite carbonate $\delta^{13}C$ values on Saipan clearly indicate that a significant number of calories came from C_4 or marine plants with very low protein concentrations. Diagenesis of apatite carbonate seems unlikely, because we found no correlation be-

tween $\delta^{13}C$ values and carbonate yields. Traditional consumption of seaweeds is not well-documented in the Marianas. Two species are, however, consumed there now and seaweeds are eaten throughout the Pacific (Abbott, 1991).

The only prehistoric staple C_4 plant known in the Marianas is sugar cane (Pollock, 1986). In highland Papua New Guinea it has been recovered from a stratum dated to 4500 BP (Daniels and Daniels, 1993). It was thus domesticated prior to the spread of Austronesian-speaking populations from the SW Pacific and was probably carried with them throughout Oceania 3500 years ago. Daniels and Daniels (1993) have compiled an impressive body of evidence that suggests that sugar cane was a staple crop in many areas of New Guinea prior to the introduction of the sweet potato 250 years ago. They note that sugar cane, unlike purified sugar, contains substances that prevent tooth decay. Therefore the low incidence of dental caries in the Marianas (Hanson, 1988, 1989, 1990, 1991, 1995) should not be considered evidence against consumption of sugar cane. Dental caries occur in very low frequencies (0–4.2%) in seven archaeological populations on Guam, Rota and Saipan. However, two populations from Saipan analyzed by Pietrusewsky and Douglas (cited in Hanson, 1995) have caries frequencies of 10.2% to 11.0%. Although sugar cane is argued to have cariostatic properties it may nonetheless explain the high caries incidence on Saipan.

CONCLUSIONS

We conclude that the isotopic analysis of collagen and apatite carbonate provide new insights into variation in proportions of marine versus terrestrial resources in ancient Chamorro diets. Most individuals had a largely terrestrial plant-based diet and consumed varied, but relatively small amounts of marine protein. Sugar cane and/or seaweeds apparently made small contributions to prehistoric human diets on Guam and Rota, but may have played an important role on Saipan.

The consumption of significant amounts of sugar cane and/or seaweeds was not anticipated and does not fit the conventional picture of prehistoric Chamorro subsistence (Butler, 1988; Pollock, 1986; McGovern-Wilson and Quinn, 1996). However, based on the results of controlled diet experiments (Ambrose and Norr, 1993; Tieszen and Fagre, 1993), this is the most parsimonious interpretation of the isotopic data. This interpretation is also supported by comparatively high caries frequencies on Saipan. Carbonate carbon isotope analysis has thus apparently revealed an important dimension of diet that was effectively invisible in collagen carbon isotope ratios. In the absence of direct archaeobotanical evidence consumption of large amounts of sugar cane on Saipan remains a hypothesis to be tested by further research. Phytolith and charred macrofloral analyses have been conducted by Pearsall (1995). Grass phytoliths were recovered but comparisons were not made with those of sugar cane. Further archaeobotanical research is warranted.

It would be premature to draw conclusions regarding dietary correlates of age, gender, health, social status and diachronic change from the small number of samples analyzed in this study. Stable isotopic analysis of many more prehistoric humans could help identify such differences and also evaluate potential relationships to changes in population density and agricultural intensification. Agricultural intensification and decline in marine resource use is documented on Rota Island at the transition to the Latte Period (Butler, 1988). This should be reflected by declining collagen $\delta^{13}C$ and $\delta^{15}N$ values through time. It would be useful to analyze a series of pre-Latte and Latte Period skeletons to provide quantitative data for this inferred dietary shift. Stable isotope analysis of human bone could be used to evaluate the role of diet in cultural evolution throughout the Pacific Islands.

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